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LOW-DOSE PRETREATMENT FOR RADIATION THERAPY

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□ In radiotherapy, a large radiation dose must be applied to both cancer and neighboring healthy cells. Recent experiments have shown that a low dose of ionizing radiation turns on certain protective mechanisms that allow a cell to better survive a subsequent high dose of radiation. This adaptive response can have important and positive consequences for radiotherapy. This paper describes a simple change in treatment procedures to make use of these beneficial effects. A low dose applied only to the healthy cells will probably produce some damage. However, it will also start the adaptive response which will yield increased protection when the large therapeutic dose is applied. The resultant immediate damage will be thereby reduced as well as the probability that the high dose therapy itself will induce a subsequent secondary cancer. After a brief historical review, the effects of a low radiation dose on a canine cancer cell line will be discussed as well as trials of the suggested pre-dose therapy on canine cancer patients undergoing standard radiation therapy.

Keywords: adaptive response, radiation therapy, radioprotection, canine cancer

INTRODUCTION

In this paper a preliminary report is presented on the results of a proposed pre-dose protocol to be applied several hours before standard radiation therapy (Blankenbecler 2005). The pre-dose is only applied to the healthy cells surrounding a tumor in order to induce a protective adaptive response. Thus the healthy cells will be preconditioned for the subsequent high dose while the cancer cells will not. The results of applying this new therapy to canine patients will be reported. Preliminary results on the genetic response of a canine cancer cell culture to low dose exposure will also be discussed. A full discussion of the research will be given in subsequent papers after all the data is analyzed (Blankenbecler, *et al.*, 2010 and Burke, *et al.*, 2010). This paper is a written version of a talk given at the Community Environmental Monitoring Program Workshop (CEMP 2009) .

The study of the cellular and molecular responses induced by a low dose of ionizing radiation can now be studied in detail using genomic assays (Brooks 2005). Earlier technology was not sensitive enough to

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directly measure the genetic modulation from very low levels of ionizing radiation (below ~ 10 cGy, roughly equivalent to several CT scans). However, the beneficial effects of a pre-dose were evident from studies of lifetime, cancer incidence and cancer latency.

Radiation therapy (RT), chemotherapy and surgical excision are common methods used for treating tumors in humans and animals (Freeman, *et al.*, 2003). The success of radiation therapy in controlling or eliminating a neoplasm is dependent on many factors (McEntee 2002). Not all tumors can be successfully treated with RT. Some tumor cells are radiation resistant due to an enhanced repair capability or an ability to resist radiation-induced DNA damage.

RT is limited by the complications and damage to the normal tissues surrounding the tumor. Thus the goal is to devise a therapy that delivers a lethal dose to the cancerous cells but limits the damage to the nearby healthy cells. This is accomplished at present by several methods including dose fractionation and radiation beam design (Freeman, *et al.*, 2003). This paper will describe another tactic that can be added to these – the use of a pre-dose to the healthy cells to utilize their adaptive response and reduce their sensitivity to a subsequent large radiation dose (Blankenbecler 2005). This effect can be used in several ways. This procedure allows the therapeutic dose to be increased, thereby improving effectiveness and reducing the number of dose fractions and treatment cost, or it can be used to reduce the side effects from the therapy by decreasing patient discomfort. The probability that the high dose therapy itself will induce a late secondary cancer may also be reduced and its latency increased.

BRIEF REVIEW

There have been many experiments studying the effects of low dose radiation. One of the earliest references is the work by Russ (1909) who showed that mice treated with low levels of radiation were more resistant against bacterial disease. Recent advances in experimental techniques, especially the ability to directly measure identifiable gene activity, have led to a new level of understanding of the response of cells to low dose radiation. The nonlinearity of the effect can be understood in terms of gene modulation, both up and down, and the time dependence of protein production.

The Department of Energy has funded a special research effort aimed at understanding the effects of low dose radiation. This program has been reviewed by A. Brooks (2005). There is a compendium of papers available on the web (Low Dose Site 2010). Most of the discussion inspired by these experiments concerns the linear no threshold hypothesis, the LNT. For a general review of the LNT see Cohen (2002).

Yonezawa, *et al.*, (1996), exposed ICR mice to 8 Gy of X-rays. Roughly 30% of the mice survived 30 days after exposure. However, when another group was "pre-irradiated" with 5 cGy several hours before the 8 Gy exposure, the survival rate increased to roughly 70%. In other experiments, low dose "pre-exposure" delayed the onset of leukemia induced by a subsequent exposure to a much larger dose of radiation. An adaptive response arising from occupational exposure was studied by Barquinero, *et al.*, (1995).

Broome and co-workers (Broome, *et al.*, 2002) administered low doses of gamma radiation (0.1 cGy-50 cGy) to human skin cell tissue several hours before exposure to a single high dose of 4 Gy gamma-radiation. Using a micronucleus formation frequency assay, they found fewer chromosomal micronuclei in cells pre-treated with low levels of radiation than in cells that received no pretreatment. They attributed the observed effects to increased efficiency of chromosomal repair in the cells pretreated with low dose radiation. It was also shown (Azzam, *et al.*, 1994a, 1994b, 1996) that pretreatment of mouse embryonic cells in tissue culture with a low dose (0.1-10 cGy) of gamma radiation reduced the rate of neoplastic transformation of these cells following a higher (4 Gy) mutagenic dose of radiation. They attributed these results to stimulation of DNA repair mechanisms by low-dose radiation exposure. Mitchel and coworkers (Mitchel, *et al.*, 2002, 2003, Mitchel 2004), in a series of studies using low dose and high dose exposures of cancer prone Trp53 heterozygous mice, demonstrated reduced rates of tumor formation and longer periods of latency in tumor development when mice were pretreated with low doses of radiation prior to subsequently receiving a high dose. However, the probability of eventual cancer formation was not reduced.

Heller (2003) reviewed the results of research conducted at the Lawrence Livermore Laboratory in which gene microarray data were used to demonstrate the adaptive effects of low level irradiation in a variety of cell systems. It was shown that a low dose modulates several hundred genes, including those involved in general cell repair. For further research results see Coleman and Wyrobek (2006) and Yin, *et al.*, (2003). In vivo experiments at U. C. Davis on healthy human skin cell plugs, Goldberg, *et al.*, (2004) have demonstrated similar, but not quite identical, results for the cellular adaptive response in these complex mixture of cell types.

Exposure experiments on rats have been carried out at the Chernobyl accident site (Rodgers and Holmes 2008). Under a variety of conditions, a pre-dose of 10 cGy was given at various dose rates up to 24 hours before a challenge dose of 1.5 Gy. The frequency of micronucleus formation was reduced by a factor of 2.6 following a pre-dose given at the highest dose rate when compared to the same 1.5 Gy challenge dose without the pre-dose.

The use of a whole body pre-dose before standard radiation therapy has been suggested and discussed (Jin, *et al.*, 2007). This technique has the disadvantage of exposing the entire body to unnecessary radiation. It also may trigger a protective adaptive response in the cancerous tissue thereby reducing the kill probability from the standard treatment. More study is needed to evaluate the full effects and potential of this protocol.

A general review of low dose effects has been given by Mitchel (2010). It is important to study and discern whether low-level exposure always constitutes a significant risk to patients, or if, in fact, under certain prescribed conditions, some exposure can be protective. In particular, the research described here is the first step in showing that the protective effect can be used to reduce the damage to healthy cells from the high exposure used in standard therapy.

CANINE MALIGNANCY

Radiation therapy (RT) is very useful in the treatment of certain oral neoplasms in dogs. Oral malignancies in dogs may originate in several source tissues. The most common types of malignancies – squamous cell carcinoma, melanoma and fibrosarcoma – have been recognized and well-studied for more than 30 years (Todoroff and Brody, 1979). In dogs, these malignancies produce a variety of clinical signs and they often show an aggressive growth pattern into soft and bony tissue. They may also metastasize to distant sites. The suggested therapy for these tumors is a combination of surgical resection/debulking and radiation therapy (RT). Many oral tumors are relatively sensitive to radiation. The head and oral cavity can be positioned to allow sharply focused radiation to be delivered, sparing other parts of the body from secondary irradiation. Veterinary radiation oncologists are experienced in treating these tumors and there are well- established and tested protocols for irradiating such cancers and measuring the side effects.

CANINE CELL CULTURE IRRADIATION

The cell culture studies were undertaken to see if exposure to low level (~0.1 Gy) γ-irradiation 24 hours prior to exposure to a higher level of γ-radiation (~2.0 Gy) would affect expression of genes in cells that might function to protect these cells from the effects of irradiation. We chose to use a stable, well-characterized neoplastic cell line for these gene modulation studies. A continuously-growing, stable canine squamous cell carcinoma cell line (SCCA 2/88) was generously donated for use by Dr. Elaine Müller and Prof. M. Suter (University of Bern Institute of Animal Pathology, Längestrasse 122 CH-3012 Bern, Switzerland). Cells were cultured in flasks and then subdivided into 25 ml. culture flasks for control, radiation exposure, and analysis.

A cobalt 60 γ-ray source was used to irradiate the cultures at a dose rate of 7 cGy/min. The first control flask was not exposed to radiation. The second flask was exposed to 10 cGy only. The third flask was exposed to 10 cGy and 24 hours later received 2.0 Gy. The forth flask was exposed to 2.0 Gy only. Cells were then harvested at selected times following "radiation therapy" (1 hour, 6 hours, 24 hours, 48 hours, and 72 hours), stabilized, and their mRNA isolated.

RNA was extracted from the irradiated tumor cells and hybridized on Canine 2.0 gene chips from Affymetrix. Assays were performed in triplicate to achieve uniformity of preparation and analysis. After quality assessment of the arrays, the probe intensities were normalized (Lim, *et al.*, 2007) . Principal components analysis and hierarchical clustering were used (Yeung and Ruzzo, 2001). Linear modeling of microarray data analysis, LIMMA, was used to detect changes in gene expression (Smyth 2005) and was used on all samples to identify genes in which there was at least a 2-fold difference in gene expression, either up-regulation or down-regulation, based on mRNA levels. Approximately 20,000 non-redundant predicted genes are assayed on the Affymetrix Canine GeneChip 2.0 Array.

At this juncture, only the 48 hour data has been analyzed and will be described here. The analysis of the complete data set will be reported later (Blankenbecler, *et al.*, 2010). Exposure to 10 cGy had little effect on tumor gene expression, two were up-regulated and one down-regulated. Not unexpectedly, a large dose (2.0 Gy) of irradiation produced substantial alterations in gene expression within 48 hours of exposure.

Comparing the control group with the 2.0 Gy group, it was found that 568 genes were up-regulated while 854 genes were down-regulated. Comparing the 10 cGy group with the 2.0 Gy group, it was found that 523 genes were up-regulated while 740 genes were down-regulated. In comparing the control group with the (10 cGy +2.0 Gy) group, it was found that 541 genes were up-regulated while 902 genes were down-regulated. However, a substantially higher number of genes, 798, are up-regulated, and a substantially lower number of genes, 509, are down-regulated, when data from the (10 cGy+2.0 Gy) Group is compared with the 10 cGy group.

There is a clear difference in gene regulation associated with low dose pretreatment of cells and high dose effects. We have identified a set of 68 genes associated with control of heat-shock proteins and DNA-repair, and then repeated LIMMA on these genes to identify changes associated with the various exposures.

CANINE PATIENT TRIALS

Canine patients with oral cancers were examined and selected for the study according to predefined criteria. Owners were informed that the

objective of this study was to determine if a low level (0.1 Gy) exposure 24 hours before a standard course of fractionated radiotherapy would affect gene expression and provide protection for the healthy cells surrounding the tumor. Informed owner consent was obtained for participation in the study. Standard veterinary and institutional animal care procedures were followed throughout the trials.

Any dog with gross squamous cell carcinoma or acanthomatous epulis was considered for the trial (Morrison 2002). Surgery and standard radiotherapy were offered to the owners of all patients. A total of 8 dogs were enrolled during the trial period. Test subjects and control subjects were selected randomly from the pool. Five dogs had mandibular tumors and 3 dogs had maxillary tumors. Biopsies were taken from all patients. All dogs had three-view thoracic radiographs prior to therapy. None of the selected patients had evidence of metastatic spread. The patients were anesthetized during all therapy sessions. Tumor dimensions were measured by calipers and the treatment fields were centered on the gross disease with 3 cm margins. The 4 patients receiving low-dose pretreatment were given a single dose of 10 cGy of 6 MV x-ray radiation and delivered to the field at a source-skin distance of 100 cm to the bolus. The tumor was shielded with preformed lead blocks placed so as to prevent exposure to the pretreatment dose.

Twenty-four hours later, the treatment course of 48 Gy in 16 x 300 cGy fractions was started on all patients. As before, the patient was anesthetized and placed in position for treatment. Biopsies were obtained from the tumor and a representative region of normal tissue in the treatment field.

Following the biopsies, the treatment field was reestablished using photographs and ink lines marked on the patient. No lead blocks were used and the entire tumor was exposed to radiation. Each treatment fraction consisted of 300 cGy delivered in a single session. Tumor response and side effects were observed daily and recorded at the end of each treatment week.

The biopsy procedure was repeated on the last day of therapy prior to administration of the final treatment fraction. Normal tissue side effects and tumor measurements were recorded. The radiation oncologist administering therapy evaluated each patient for the presence or absence of therapeutic effects on the tumor and for the presence or absence of acute radiation side effects. A pre-determined scale for grading the severity of acute side effects was used to record data on each patient during the course of therapy. Observations of patients and objective assessment of condition occurred on a twice-daily basis throughout the course of therapy, and then at prescribed intervals following therapy. The importance of photographic documentation following therapy was recognized in the assessment of side effects, tumor growth, and patient progress.

A standard veterinary pre-determined scale (0=none, 1=minimal, 2=moderate, 3=severe effects) was used for grading the severity of the side effects for each patient during the course of the therapy. The observed scores at the conclusion of the treatment for skin effects were (1,2,1,2: average 1.5) for the pre-dose group and (3,1,3,2: average 2.25) for the control group. The scores for side effects on the mucous membrane were (1,2,2,2: average 1.75) for the pre-dose group and (3,2,2,2: average 2.25) for the control group. Based on this limited data, the scoring of lesions of the oral cavity following the fractionated RT showed a decrease in severity of distressful side effects in those dogs that had a low dose pretreatment as compared to the control group. Experiments have not yet been performed that test the effect of an increase in the per fraction dose following the pre-dose treatment. If these prove to be effective, then the decrease in the number of fractions and hence the number of times that the dogs must be sedated could lead to substantial benefits in terms of health, cost, safety, and recovery time.

CONCLUSIONS

Results of this small pilot study appear to indicate a cytoprotective effect of low dose radiation (0.1 Gy) pretreatment of normal tissues before standard fractionated therapy for oral tumors in dogs. These clinical observations support the studies discussed above on canine squamous cell carcinoma tissue cultures. Canine neoplasms can be a useful model for human cancers since dogs suffer from essentially the same types of natural cancers as humans but with quite different frequencies that are also highly breed dependent, suggesting a genetic connection.

At the same time, these results are cautionary. More work is needed to study the balance of effects between cytoprotection of normal tissues and the RT effects on tumor tissues. In particular, the time dependence of the adaptive response needs to be better determined in order to plan an optimized treatment. The adaptive response at 24 and 48 hours is well enough known that the low dose pretreatment could be applied with confidence for radiotherapy protocols that use few fractions, such as gamma knife or similar technologies. With a better understanding of the time dependence, optimum protocols could be developed for multifraction treatment schemes. The adaptive response of different cell types also needs to be studied. However, the protective effect of the adaptive response when a low dose is applied to the entire animal shows that the response should be quite universal among cell types.

Taken together, this initial in vitro and in vivo work suggests that protocols can be developed and optimized that will minimize the acute and late associated side effects from RT. This could substantially change how radiotherapy is planned and performed, and provide methods to more successfully treat patients.

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